SCIENTIFIC LETTER

Association between aldosterone synthase (CYP11B2) gene polymorphism and left ventricular volume in patients with dilated cardiomyopathy

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Aldosterone has an effect on the genesis and progression of cardiac remodelling. Aldosterone biosynthesis is regulated by a key enzyme, aldosterone synthase (CYP11B2). In humans, several frequent polymorphisms have been described in the promoter of this CYP11B2 gene.[1] In particular, T-344C polymorphism involves a T/C substitution in a putative binding site for steroidogenic transcription factor SF-1, and a fourfold increase in binding of SF-1 to the -344C allele has been shown in vitro.[1]

We compared retrospectively the left ventricular (LV) characteristics, haemodynamic parameters, and biochemical data with the CYP11B2 genotype in patients with idiopathic dilated cardiomyopathy (DCM). We further conducted a case–control study to elucidate whether this polymorphism represents a susceptibility gene to DCM.

METHODS

Two hundred and one DCM patients and 183 age and sex matched control subjects were enrolled in the study. All the DCM patients underwent left ventriculography (LVG) and coronary angiography to exclude coronary artery disease and segmental LV wall motion abnormality. Consecutive patients were recruited from the inpatients of Kobe University Hospital (Kobe, Hyogo) from January 1995 to June 2001 or Himeji Cardiovascular Center (Himeji, Hyogo) from January 1995 to February 1999. Healthy control subjects were recruited from company employees (Akashi, Hyogo) and completed a series of questionnaires, physical examinations, routine laboratory tests, chest radiographs, and ECGs. Control subjects with cardiomegaly, ECG abnormalities, and documented heart diseases were excluded from this study. All subjects enrolled were Japanese and written informed consent was obtained. The study design was approved by the ethics committee of Kobe University and Himeji Cardiovascular Center.

Data on blood pressure, New York Heart Association (NYHA) functional class, medication status, and haemodynamic and left ventriculographic parameters were assessed only at the patients' first admission. LV end diastolic and end systolic volume and ejection fraction were measured by the area–length method in the 30° right anterior oblique projection by a blinded experienced observer. Haemodynamic parameters were also obtained. Patients were clinically stable for at least one week before the catheterisation study. Plasma aldosterone concentration and renin activity were determined by radioimmunoassay early in the morning after at least 30 minutes of rest in supine position in 102 DCM patients who were in stable heart failure for at least for five days. All medications were withheld for at least 12 hours before the catheterisation and biochemical studies. Dietary salt intake was restricted to 6–8 g a day.

Genomic DNA was extracted from peripheral blood. Genotyping for the CYP11B2 T-344C polymorphism was performed as previously described.[2] Data are presented as mean (SEM). The differences between the groups (DCM and control, or TT and TC+CC genotype) were analysed by the unpaired Student's *t* test or Mann-Whitney U test, and by Fisher's exact test or χ^2 analysis for discrete variables. We also performed multivariate regression analysis. Probability values of $p < 0.05$ (two tailed) were considered to indicate significance.

RESULTS

The allele frequencies in the controls and DCM patients were in Hardy-Weinberg's equilirium. There was no significant difference in the allele distribution between the two groups (T allele frequencies: 0.67 in controls and 0.66 in DCM patients, respectively).

We compared clinical characteristics including both haemodynamic and LVG parameters between two genotype groups (TT group (n = 87) and TC+CC group (n = 114)) to clarify the contribution of the C allele to the progress of cardiac remodelling. Moreover, we combined TC and CC based on the previous finding that the presence of the C allele for this polymorphism was associated with a co-dominant increase in aldosterone concentrations of 22% and 44% in heterozygotes and homozygotes, respectively.[3] Table 1 shows the clinical parameters according to genotype. No significant differences between the two groups were found with respect to age, sex, body surface area, body mass index, systolic and diastolic blood pressure, prevalence of hypertension, NYHA functional class, alcohol consumption, or use of medication (angiotensin converting enzyme (ACE) inhibitor, angiotensin II type 1 receptor blocker (ARB), diuretics, digitalis, and β blocker). LV end diastolic volume index as measured by LVG was significantly larger in the TC+CC group than in TT group (TT *v* TC+CC: 132.1 (3.9) *v* 146.0 (4.4) ml/m^2; $p < 0.05$). LV end systolic volume index tended to be larger in the TC+CC than in the TT group (TT *v* TC+CC: 87.1 (3.7) *v* 100.0 (4.2) ml/m^2; $p = 0.069$). The systolic function estimated by ejection fraction was not significantly different between the two groups (TT *v* TC+CC: 35.6 (1.2)% *v* 33.5 (1.1)%). Regarding haemodynamic parameters, the cardiac index in the TC+CC group tended to deteriorate (TT *v* TC+CC: 2.91 (0.07) *v* 2.79 (0.07) l/min/m^2; $p = 0.14$). However, other parameters including pulmonary capillary wedge pressure (TT *v* TC+CC: 9.5 (0.6) *v* 10.3 (0.7) mm Hg) and LV end diastolic pressure (TT *v* TC+CC: 13.2 (0.8) *v* 12.9 (0.6) mm Hg) did not differ between the two groups.

Abbreviations: ACE, angiotensin converting enzyme; ARB, angiotensin II type 1 receptor blocker; DCM, dilated cardiomyopathy; LV, left ventricular; LVG, left ventriculography; NYHA, New York Heart Association

Table 1 Clinical characteristics of dilated cardiomyopathy patients according to CYP11B2 genotype

	TT (n=87)	TC (n=91)	CC (n=23)	TC+CC (n=114)	p Value
Age (years)	52.3 (1.4)	54.8 (1.3)	52.7 (2.7)	54.4 (1.2)	NS
Sex (male/female)	(71/16)	(69/22)	(16/7)	(85/29)	NS
BP systolic (mm Hg)	124.6 (1.9)	121.3 (1.5)	125.0 (4.5)	122.1 (1.5)	NS
BP diastolic (mm Hg)	76.5 (1.2)	74.2 (1.0)	76.1 (2.8)	74.5 (1.0)	NS
Hypertension (n)	24	24	7	31	NS
NYHA (I+II/III+IV)	(64/23)	(59/32)	(16/7)	(75/39)	NS
Medication					
ACEI or ARB (n)	42	41	9	50	NS
Diuretics					
Furosemide (n)	48	62	15	77	NS
Spironolactone (n)	26	33	9	42	NS
Digitalis (n)	35	49	12	61	NS
β Blocker (n)	6	7	2	9	NS
LVEDVI (ml/m^2)*	132.1 (3.9)	145.0 (5.0)	150.0 (9.5)	146.0 (4.4)	<0.05
LVESVI (ml/m^2)*	87.1 (3.7)	98.6 (4.7)	105.5 (9.9)	100.0 (4.2)	0.069
EF (%)*	35.6 (1.2)	33.9 (1.2)	32.2 (3.1)	33.5 (1.1)	NS
	TT (n=46)	TC (n=43)	CC (n=13)	TC+CC (n=56)	p
PRA (ng/ml/hour)*	4.39 (0.96)	4.82 (1.09)	4.11 (1.52)	4.63 (0.93)	NS
Aldosterone (pg/ml)*	113.7 (9.5)	140.2 (15.1)	162.5 (20.2)	145.4 (12.5)	<0.05

Data presented as mean (SEM).
The difference between TT and TC+CC was analysed by the unpaired Student's *t* test or Mann-Whitney U test, and by Fisher's exact test for discrete variables. *Mann-Whitney U test was used.
ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II type 1 receptor blocker; BP, blood pressure; EF, ejection fraction; LVEDVI, left ventricular end diastolic volume index; LVESVI, left ventricular end systolic volume index; NYHA, New York Heart Association functional class; PRA, plasma renin activity.

Multiple regression analyses revealed that TC+CC genotype and systolic blood pressure were independent statistical predictors of LV end diastolic volume index.

Although there were no significant differences in potassium concentration and plasma renin activity between the two groups, plasma aldosterone concentration in the TC+CC group was significantly higher than in the TT group (TT *v* TC+CC: 113.7 (9.5) *v* 145.4 (12.5) pg/ml; $p < 0.05$). Regarding the frequency of medication such as frusemide (furosemide), spironolactone, ACE inhibitor, or ARB, no significant differences were observed between the two groups in the biochemical study (data not shown).

Even after the exclusion of patients receiving spironolactone, the concentration of plasma aldosterone in the TC+CC group was significantly higher than in TT group (TT *v* TC+CC: 107.1 (9.5) *v* 138.4 (15.3) pg/ml; $p < 0.05$).

DISCUSSION

We have shown that the TC+CC genotype in the CYP11B2 was significantly associated with larger LV volume in DCM. However, the genotype distribution in DCM was not statistically different from that in controls, implying this polymorphism cannot represent a susceptibility gene to DCM.

The prevalence of the CC genotype in Europeans is twice that found in Japanese,[2–5] suggesting ethnic differences may exist regarding this genotype. Recently, Tiret and colleagues reported that T-344C polymorphism was not associated with severity of DCM.[5] This discrepancy may be due to either the difference of methods used for the evaluation of the disease severity, or ethnic differences. We assessed the LV volume only with LVG in order to minimise the methodological bias, whereas Tiret and colleagues[5] analysed the combined data obtained by LVG, radionucleotide angiogram, or echocardiogram.

Plasma aldosterone concentration in the TC+CC group was higher than in TT group. Aldosterone concentrations are dependent on the degree of compensation, medications, and underlying pathological conditions in heart failure. All venous sampling was carefully undertaken when patients were in stable heart failure for at least five days and there were no differences in medication, including ACE inhibitor, ARB, and diuretics, in sodium–potassium balance, and in blood pressure between two groups.

The study population was relatively small and limited to Japanese. Thus care should be exercised in the extrapolation of these findings to other populations. A survival selection bias might have been introduced because of the retrospective study design. Thus, there is a need to conduct a more extensive and prospective study.

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